The present invention is further illustrated by the following examples, which are not intended to be limiting.

EXAMPLE I

The following example describes the conditions for formation of a Protein A-I^{125}/rabbit anti-ferritin IgG complex and its use in a radioimmunoassay for ferritin.

Complex Formation: Rabbit anti-ferritin IgG, isolated from whole sera, of a desired concentration in 0.02 M phosphate buffer (pH 7.4, 0.1% BSA, 0.1% NaN$_3$) is combined with a solution of Protein A-I^{125} (obtained from Amersham, Arlington Heights, Ill.) of the desired concentration in 0.02 M phosphate buffer (pH 7.4, 0.1% BSA, 0.1% NaN$_3$) and incubated for one hour at 37° C. For this example, three such solutions were prepared with molar ratios of rabbit anti-ferritin IgG to Protein A-I^{125} as shown in Table I.

Radioimmunoassay

The procedure employed polystyrene bead (0.25 inches diameter) supported rabbit anti-ferritin (IgG) antibody obtained from a ferritin radioimmunoassay kit marketed by Ramco Laboratories, Houston, Tex. To a set of test tubes were added aliquots (10 ul) of ferritin standard solutions with ferritin concentrations as shown in Table I. The liquid volumes in each tube were made up to 210 ul with the addition of 200 ul of 0.02 M phosphate buffer (pH 7.4, 0.1% BSA, 0.1% NaN$_3$). A polystyrene bead was added to each tube, and the tubes were incubated for three hours at 37° C. The solid support was then washed three times with the 0.02 M phosphate buffer. To each tube was then added an aliquot (200 ul) of a solution containing the rabbit anti-ferritin IgG/Protein A-I^{125} complex. The tubes were incubated for one and one-half hours at 37° C. and the solid support was washed three times with the 0.02 M phosphate buffer. The polystyrene beads were individually transferred to clean test tubes and placed in a gamma counter and the bound radioactivity measured. The results are shown in Table I. An analysis of this data indicates that for those tubes having a soluble IgG/PA I^{125} ratio of 25,000:1, the sensitivity was limited and for tubes having a soluble IgG/PA I^{125} ratio of 250:1, background was excessive and a non-linear response was observed. For those tests having a soluble IgG/PA I^{125} ratio of 2500:1, a substantially linear response was obtained up to a concentration of 600 ng/ml with non-interferring background levels.

EXAMPLE II

The experiment of Example I was repeated in all essential details except that soluble rabbit anti-ferritin IgG concentrations were 300 micrograms per milliliter, 60 micrograms per milliliter, 6 micrograms per milliliter and 3 micrograms per milliliter, and an alkaline phosphatase-protein A conjugate purchased from Zymed Laboratories, Burlingame, Calif., was substituted for radiolabeled protein A and used at a dilution of 1:1000 in 0.02 M phosphate buffer (pH 7.4, 0.1% BSA, 0.1% NaN$_3$). Following the second antibody/protein A-alkaline phosphatase complex incubation period and wash sequence, the solid supports were transferred to clean tubes and incubated at 37° C. in 0.1 M glycine, pH 10.4, containing 1 mM magnesium chloride and 2 mg/ml p-nitrophenylphosphate (200 ul). The enzyme reaction was terminated after 20 minutes of incubation with 1.0 milliliters of 0.5 molar sodium hydroxide, and optical

density measurements were made at 405 nm. The results are shown in Table II. Again, low concentrations of soluble IgG produced high levels of background signals, and high concentrations of soluble IgG resulted in limited sensitivity. A soluble IgG concentration of 60 micrograms per milliliter resulted in linear assay results having low levels of background interference.

EXAMPLE III

The experiment of Example I was repeated in all essential details except that enzymatically digested rabbit anti-ferritin IgG was used as first antibody on polystyrene beads. Soluble rabbit anti-ferritin IgG/Protein A-I^{125} complexes were formulated to yield the molar ratios given in Table III. The results of the experiment are also shown in Table III. An analysis of this data shows that improved sensitivities and linear responses are achieved by proper adjustment of the soluble rabbit anti-ferritin IgG/labeled protein A molar ratio. When the assay is conducted with enzyme-digested first antibody, sensitive, linear results are obtained with substantially lower molar ratios (approaching stoichiometric unity) of second antibody to labeled Protein A as compared to assays employing whole first antibodies.

EXAMPLE IV

The experiment of Example II was repeated in all essential details except that enzyme-digested rabbit anti-ferritin IgG was used as first antibody on polystyrene beads. Soluble rabbit anti-ferritin IgG concentrations were as shown in Table IV.

TABLE I

	Bound Protein A (I-125) on Supported Rabbit Anti-Ferritin IgG		
	CPM (Avg.)		
Ferritin Std.	Rabbit anti-ferritin IgG/PA-I^{125}(Molar)*		
(ng/ml)	25,000/1	2,500/1	250/1
0	175	516	1193
6.0	157	606	1725
20.0	209	695	1882
60.0	369	1051	2558
200.0	620	1964	3898
600.0	1166	3189	4725
2000	2003	—	5169

*A constant Protein A-I^{125} concentration (3.2 ng/ml) was used throughout. Rabbit anti-ferritin IgG concentrations were 300 ug/ml, 30 ug/ml and 3 ug/ml. These correspond to molar IgG/Protein A I^{125} ratios of 25,000/1, 2500/1 and 250/1, respectively.

TABLE II

	Bound Protein A-Alkaline Phosphatase on Supported Rabbit Anti-Ferritin IgG		
	Optical Density$_{405}$		
Ferritin Standard	Rabbit anti-ferritin IgG (ug/ml)		
(ng/ml)	300	60	6	3
0	0	0	.025	.060
6.0	0	0	.028	.072
20.0	0	.006	.062	.096
60.0	.002	.026	.108	.143
200.0	.014	.088	.190	.241
600.0	.065	.176	.301	.318

TABLE III

	Bound Protein A (I-125) on Supported Rabbit Anti-Ferritin F(ab')$_2$		
	CPM (Avg.)		
Ferritin Standard	Rabbit anti-ferritin IgG/PA-I^{125} (Molar)*		
(ng/ml)	597/1	119/1	60/1
0	800	776	785